Report of Proceedings

EASTERN EXPERIMENT STATION COLLABORATORS' CONFERENCE ON PROTEINS

October 25 and 26, 1960

Conference was held at the Eastern Utilization Research and Development Division with representatives from the State Agricultural Experiment Stations, universities, and the U. S. Department of Agriculture participating.

This report summarizes the discussions of the various speakers during the conference. If further details regarding any particular subject are desired, they may be obtained by communicating with the person concerned (see appended list of names and addresses).

Eastern Utilization Research and Development Division
Agricultural Research Service
U. S. Department of Agriculture
Philadelphia 18, Pennsylvania

PROGRAM 960877

Tuesday, October 25

9:30 a.m.	Introductory Remarks	P. A. Wells, Director, Eastern Utilization Research and Development Division
9:40 a.m.	Bovine Muscle Proteins	Robert J. Gibbs, Eastern Utilization Research and Development Division, Beltsville, Maryland
10:40 a.m.	Studies on the Separation and Composition of Wheat Gluten Proteins	John H. Woychik, Northern Utilization Research and Development Division, Peoria, Illinois
11:40 a.m.	Soluble-Fraction Ribonucleic Acids and Protein Synthesis	Robert W. Holley, U. S. Plant, Soil & Nutrition Laboratory, ARS, and Cornell University, Ithaca, New York
12:30 p.m.	LUNCH	
2:00 p.m.	Structure of Proteins - a general talk	Walter J. Kauzmann, Princeton University Princeton, New Jersey
3:00 p.m.	The Internal Structure of β-Lactoglobulin in Solution	Charles Tanford, Duke University, Durham, North Carolina
4:00 p.m.	Structural Studies on α-Lactalbumin	Leopold Weil, Eastern Utilization Research and Development Division

Wednesday, October 26

9:00 a.m.	Soluble Collagen	Leo D. Kahn, Eastern Utilization Research and Development Division
10:00 a.m.	Chromatographic Separation of Proteins and Peptides	Norbert J. Hipp, Eastern Utilization Research and Development Division
11:00 a.m.	Amino Acid Analysis of Milk Proteins	William G. Gordon, Eastern Utilization Research and Development Division
12:00 Noon	LUNCH	
1:30 p.m.	Allergenic Proteins of Oilseeds	Joseph R. Spies, Eastern Utilization Research and Development Division Washington, D. C.

30 p.m. LABORATORY DEMONSTRATIONS AND VISITS

Automatic amino acid analysis.

Starch gel electrophoresis.

Peptide separation by two-dimensional electrophoresis - chromatography. Column chromatography on cellulose ion-exchangers.

Low-voltage paper electrophoresis - Durrum cell.

Enzymic degradation of proteins for determination of amino acid sequence.

Electrophoresis - diffusion apparatus (Spinco Model H).

High-voltage paper curtain electrophoresis.

INTRODUCTORY REMARKS

by

P. A. Wells
Eastern Utilization Research and Development Division

Dr. Wells described the procedures by which the topic for the annual collaborators' conference is selected and collaborators appointed from the various State Experiment Stations of the Eastern Region. This year the Station Directors selected the subject of protein chemistry for this conference. Although not always true, this year's collaborators' conference is limited to representatives of the States and the U. S. Department of Agriculture. Proceedings of the present conference will be published containing five- to six-hundred word abstracts of each paper. Each conferee will receive one copy of the proceedings, but extra copies can be obtained on request. Most of Wednesday afternoon will be devoted to laboratory visits and demonstrations but there will be no escorted tours, the conferees being free to visit any or all of the demonstrations listed depending on their respective individual interests.

BOVINE MUSCLE PROTEINS

by

Robert J. Gibbs
Eastern Utilization Research and Development Division

Among the proteins in meat muscle tissue, myosin is the most abundant and has certain unique properties. First of all, myosin is a contractile protein and is primarily responsible for maintaining the structure of the muscle myofibrils and for performing the contractions and relaxations by which muscles do work. Also, myosin contains an important ATPase (adenosinetriphosphatase) enzyme activity, which helps control the transformation of chemical energy to physical energy or work.

When meat undergoes rigor, striking changes occur, one of which is that myosin undergoes an irreversible alteration which greatly reduces its solubility. The tenderness, juiciness, and other quality factors of meat and meat products are, to a large degree, related to this and other unique properties of myosin.

In the Meat Laboratory, a new, more rapid process has been developed for the isolation and purification of myosin, giving a five-fold, or better, increase in yield. Although bovine myosin is homogeneous in the ultracentrifuge when freshly prepared, it both fragments and builds aggregates simultaneously on storage in the cold. It is a long, thin, fibrillar protein, with a tightly helical configuration, as shown by various physicochemical measurements. Titration data indicate its unusual buffering properties between pH 5 and 8, the pH zone of greatest significance for the handling of meat itself.

On treatment with trypsin, bovine myosin rapidly splits to give two protein-sized fractions (light, or L-meromyosin, and heavy, or H-meromyosin). Structurally, the meromyosins give important clues to the configuration of myosin, for each inherits certain of the unique properties of the parent myosin. Thus, H-meromyosins are inherently unstable and have ATPase activity, whereas L-meromyosin resembles myosin in solubility and viscosity behavior.

In many respects, the bovine and lamb muscle proteins are qualitatively similar to those found in rabbit muscles. Between species, however, myosin, the meromyosins, tropomyosin, actomyosin, and actin differ quantitatively in sedimentation rate, diffusion constant, viscosity behavior, titration, optical rotatory dispersion, solubility, and enzymatic behavior.

Knowledge of the mechanism whereby meat holds and absorbs water is of great importance, not only to derive information about the structure of meat, but to determine the structural basis of the binding forces in cured meats, especially in sausage products. The overall effects of pH and ionic strength on water retention in meat have been found to conform generally with the mechanism of fiber swelling, in accord with the Donnan membrane theory. Non-protein nitrogenous compounds and the water-soluble muscle proteins are not critical in this matter; but, a marked increase in water-holding capacity is accomplished by treatments with solutions which tend to extract myosin. In connection with this important effect, it remains to be determined whether this deviation from Donnan theory is a property of the muscle fibers, or whether it results because of the removal of the myosin.

References

(1) Gibbs, R. J., A. J. Fryar, C. Lockett, and C. E. Swift. Bovine muscle proteins. Federation Proc. 17, 288 (1958).
(2) Fryar, A. J., and R. J. Gibbs. Bovine muscle proteins. II. Heterogeneity in H-meromyosin preparations. Arch. Biochem. Biophys. 88, 177 (1960).
(3) Sulzbacher, W. L., R. J. Gibbs, C. E. Swift, and A. J. Fryar. Protein investigations at the USDA Beltsville Meat Laboratory. Proc. 12th Res. Conf. AMIF, Bull. 61 (1960).
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STUDIES ON THE SEPARATION AND COMPOSITION OF WHEAT GLUTEN PROTEINS

by
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Peoria, Illinois

The exclusiveness of wheat as a breadmaking cereal is accounted for by the special and distinctive characteristics of a protein substance intermixed with the starchy endosperm of wheat grain. It is only by virtue of the unique properties of this protein material that the carbon dioxide produced during dough fermentation is retained in a manner which provides the familiar porous and spongy structure of bread. This substance is recognized as the gluten protein of wheat.

The fact that wheat is presently regarded as a serious surplus commodity has stimulated considerable inquiry into the potentialities of gluten for its application to nonfood industrial utilization. However, towards this end, gluten presents some serious disadvantages, from both a technical and economic standpoint. Realization of these problems, together with the role of gluten

in the baking industry, has been responsible for the numerous and persistent scientific investigations of the fundamental structure, properties, and behavior of the wheat gluten protein.

Although wheat gluten can be separated into the classical fractions of "gliadin" and "glutenin", the question of molecular heterogeneity of wheat gluten has only recently been resolved. Through electrophoresis in buffers of low pH (3.1), Jones and coworkers (1) were able to demonstrate the presence of four major (alpha-1, alpha-2, beta, and gamma) and one minor (ω) component. Comparison of glutens obtained from flours having excellent baking qualities and those having poor quality indicated a similarity both in electrophoretic composition and relative concentrations. Significant differences in the electrophoretic patterns of glutens from durum wheats (used in the manufacture of spaghetti and macaroni) and the bread wheats were observed.

Isolation of individual electrophoretic components was achieved through the use of precipitation and chromatographic techniques. Alpha-1 was obtained by precipitation, either through increasing the pH or the ionic strength (1). Isolation of the other components required ion exchange chromatography on carboxymethylcellulose (2) using discontinuous elution with buffers of increasing hydrogen ion concentrations. Alpha-1 gluten was selectively retained by the carboxymethylcellulose and required the use of 8 M urea for its desorption.

Fundamental studies of the isolated components revealed differences in amino acid composition (3) which established the individuality of the isolated components. The high content of glutamic acid and proline (44 and 20 g./16 g. nitrogen, respectively) together with a low basic amino acid content characterizes the gluten components. Evidence obtained indicated that essentially all of the dicarboxylic amino acids are present in gluten as their amides.

Further investigation of molecular heterogeneity by zone electrophoresis in the presence of 3 M urea (4) utilizing starch gels revealed the presence of eight components, three of which were not previously detected in whole gluten by the Tiselius method. This provides further evidence for the superior resolving power of starch gels over "free" electrophoresis.

Molecular weights of the isolated components were determined (5) using the approach to sedimentation equilibrium technique of Archibald as extended by Klainer and Kegles. The following molecular weights were obtained: alpha-1, 1.5 - 3 million; alpha-2, 200,000; beta, 42,000; and gamma, 47,000. The major portion of the viscoelastic properties of gluten resides in the high molecular weight alpha-1 component. Further investigation revealed that it can be oxidatively cleaved to yield a basic unit with a molecular weight of 21,000 (6). It appears therefore, that the alpha-1 component is composed of several of these low molecular weight units linked through disulfide bonds to yield a polymer with a molecular weight range in the millions.

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SOLUBLE-FRACTION RIBONUCLEIC ACIDS AND PROTEIN SYNTHESIS

by
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Present concepts of the biosynthesis of proteins were reviewed. (For a recent review, see (1).) According to these concepts, the initial reactions in the biosynthesis of proteins are believed to be those shown in the accompanying equations:

$$\text{Enzyme}_{1\text{-}20} + \text{Amino Acid}_{1\text{-}20} + \text{Adenosine Triphosphate} \rightleftharpoons$$

$$\text{Enzyme}_{1\text{-}20} - \text{Amino Acyl}_{1\text{-}20} - \text{Adenosine Monophosphate} + \text{Pyrophosphate}$$

$$\text{Enzyme}_{1\text{-}20} - \text{Amino Acyl}_{1\text{-}20} - \text{Adenosine Monophosphate} + \text{RNA}_{1\text{-}20} \rightleftharpoons$$

$$\text{Enzyme}_{1\text{-}20} + \text{Amino Acyl}_{1\text{-}20} - \text{RNA}_{1\text{-}20} + \text{Adenosine Monophosphate}$$

In these reactions the different amino acids are activated by specific amino acid-activating enzymes and then are transferred to specific soluble-fraction ribonucleic acids (RNAs). It is believed that the RNAs then transfer the specific amino acids to their sites in the "template" for protein synthesis. The soluble-fraction RNAs are of great interest to chemists because of their role in protein synthesis, because it is anticipated that studies of their structures will furnish clues for the coding problem - the problem of how nucleic acid structure controls protein structure - and because these RNAs furnish the chemist with his first opportunity to determine the complete structure of a biologically active nucleic acid.

Studies of the separation of the different amino acid-specific soluble-fraction RNAs by countercurrent distribution and by partition chromatography were described. It was shown that certain of the RNAs have quite different partition coefficients in a two-phase solvent system composed of concentrated pH 6 phosphate buffer, isopropanol and formamide, and can be separated quite easily using this system (2-5).

References

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STRUCTURE OF PROTEINS - A GENERAL TALK

by
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This discussion dealt with two topics:

1. Intramolecular bonding in proteins, especially hydrophobic bonds. Nature of hydrophobic bonds - a result of the tendency of non-polar side chains to escape from the aqueous phase. Polypeptide chain conformations which bring non-polar side chains into contact with each other will therefore tend to be more stable than other conformations. Attempts to estimate the thermodynamics of the bond reveal that it is probably stabilized almost entirely by an entropy effect, and not an energy effect. Probable role of Frank-Evans "icebergs"(1).

2. Volume changesin protein reactions. Method of measurement (Lindestrøm-Lang dilatometers). Outline of factors which influence the volumes of protein molecules in aqueous solutions: (a) Large increase in volume associated with formation of hydrophobic bonds. (b) Large effects of electrostriction about charged groups. Detailed study of volume changes of proteins and small molecular analogues on addition of acid or base reveals that the carboxylate ions of proteins appear to have a normal environment. The amino groups, however, are appreciably different in their volume changes in proteins and in small molecules. This abnormality of amino groups is especially pronounced in wool and hair.

The volume change in the helix-coil transition of synthetic polypeptides resembles that in protein denaturation (the helix appears to be less compact by about 1 cc per 100 gm. of polymer). Anomalous effects, not yet understood, are observed in the combination of polymers such as polymethacryclic acid and polyvinyl amine, with acids and bases (2).

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THE INTERNAL STRUCTURE OF β-LACTOGLOBULIN IN SOLUTION

by
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The work of Townend and Timasheff (1) at the Eastern Regional Laboratory and of Drs. Nozaki, Bunville and De at my own laboratory (2), (3), has led to the identification of at least eight distinct configurations for the protein

β-lactoglobulin, as summarized in the following Table. N is the stable configuration of the isoelectric protein, S is formed at low pH, T has a narrow range of stability at low temperature near pH 4.65, R is formed from N near pH 7.5, H is the stable form in 70% to 80% aqueous dioxane and other organic solvents of weak hydrogen-bonding capacity, U is the stable form in aqueous formamide or urea and also occurs as intermediate in the reaction N or S $\longrightarrow$ H. The interconversion of these six configurations is reversible, and the thermodynamic parameters $\triangle$ F°, $\triangle$ H°, and $\triangle$ S° for each conversion may be determined. Unfortunately, no obvious interpretation of the values of these parameters exists at present.

Configuration D is formed by a slow irreversible reaction in alkaline solution. It may be converted to the new compact form D' in acid solutions.

CONFORMATIONS OF β-LACTOGLOBULIN

		Polypeptide Chains	$[\eta]$ cc./g.	$[\alpha]_D$	b°
N	NATIVE	2	3.5	-28°	-70°
S	SINGLE CHAIN	1	3.5 (a)	-30°	-70°
T	TETRAMER	8	- (a)	-18°	-70°
R	REFOLDED	2	4.0	-54°	-80°
U	UNFOLDED	1	12 (b)	-100°	-70°
H	HELICAL	1	12 (c)	-5°	-450°
D	DENATURED	?	(12) (d)	-100°	-50°
D'	———	?	4.5	-60°	-

A value of $[\eta]$ near 4.0 corresponds to a compact, close to spherical particle. A value of 12 could correspond to a rigid rod or a random coil. The optical rotatory constants $[\alpha]_D$ and b° have the following values for known conformations: α-helix + 10° and -650°, random coil -100° and 0°. Thus, U and D correspond closely to random structure and H to about 70% helical structure.

(a) Not measured, but probably compact.
(b) Probably variable, indicative of random coil.
(c) Probably rigid rod.
(d) Variable: depends on pH and ionic strength, definitely indicative of at least partially random coiling.

References

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STRUCTURAL STUDIES ON α-LACTALBUMIN

by
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Eastern Utilization Research and Development Division

α-Lactalbumin was first isolated by Sørensen (1) in 1939 and further characterized by Gordon and associates (2). Sedimentation studies have shown that this protein has the molecular weight of 15,500. Since cleavage of the four disulfide bonds present in α-lactalbumin did not produce a lowering of the molecular weight, it was concluded that this protein consists of a single chain. The single-chain nature of α-lactalbumin was also supported by the use of Sanger's procedure, showing that this protein contains only one mole of N-terminal glutamic acid. Similarly, by the use of carboxypeptidase, a very rapid hydrolysis of only one mole of C-terminal leucine could be observed.

α-Lactalbumin, deprived of its C-terminal leucine by the action of carboxypeptidase, retained its ability to crystallize into well-formed needle-like protein. Since the action of carboxypeptidase on α-lactalbumin was confined to the cleavage of one mole of C-terminal leucine, the possibility arose that the penultimate amino acid which could act as a barrier for this enzyme might be arginine or lysine. This problem was solved by the use of protaminase (an enzyme specific for C-terminal arginine and lysine) which showed that the next amino acid cleaved by this enzyme was lysine. By the alternate use of carboxypeptidase and protaminase, the following C-terminal primary structure of α-lactalbumin has been established:

Phe. - Lys.- Leu.- Glu.- Glu.-NH_2 His.- Ala.) Ileu.- Val.- Tyr.- Thr.-Lys.-Leu.-COOH
|
Tyr.
|
Gly.- Ser.- Asp. NH_2 Try.) - - - - - - - - - Glu.-NH_2

The amino acids listed in parentheses do not necessarily represent the sequence but only their presence in this portion of the protein molecule.

During the tryptic digestion of α-lactalbumin in addition to the formation of peptide fragments, the liberation of stoichiometric amounts of free lysine and leucine has been observed. In accordance with the specificity requirement of trypsin, the first might be due to a lysyl-lysyl or arginyl-lysyl sequence within the protein molecule, while the formation of free leucine can be only ascribed to the tryptic cleavage of the lysyl-leucine bond at the C-Terminal end of the protein.

Cleavage of disulfide bonds of α-lactalbumin by the sulfite method resulted in a moderate increase of optical rotation from -60° to -69°. The equal marked increase in optical rotation (-107°) in 8 M guanidine solution, and their reversal upon removal of guanidine by dialysis, to the original values for both proteins might be interpreted that the forces responsible for the secondary structure of α-lactalbumin are due chiefly to peptide hydrogen bonds, while the disulfide bonds represent a lesser contributing factor. The fact that the reduced viscosity of the native as well as that of the S-sulfo-α-lactalbumin (in the latter the disulfide bonds were cleaved) was not affected by increasing temperature would indicate a remarkable stable tri-dimensional structure of this protein.

References

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(2) Gordon, W. G., and Ziegler, J., Archiv. Biochem. Biophys., 57, 80 (1955).

SOLUBLE COLLAGEN

by
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The first requisite in the study of the solution properties of collagen is a homogeneous preparation. This is difficult to achieve, not only because of the problem of isolation, but also because repeated precipitation and dissolving yields a progressively diminished concentration of collagen in solution. After each precipitation, the collagen that has been reconstituted is not completely soluble.

Salting-out curves of collagen in solutions of phosphate buffer at alkaline pH and also in solutions of ammonium sulfate have been prepared and these show the expected solubility peak at an optimum concentration of electrolyte. However, when collagen reconstituted from a solution of definite buffer composition is placed in contact with a fresh portion of the same buffer all of the collagen does not redissolve, and this effect becomes more pronounced as the ionic strength of the buffer is increased. Thus, when the collagen used in preparing a salting-out curve is redissolved each subsequent salting-out curve shows a lower concentration of collagen at each value of buffer concentration. The collagen that does not redissolve presents the same appearance under electron microscope examination as that which does dissolve.

A study of the effect of solution history on the structure of reconstituted collagen fibrils was carried out using citrate buffer, phosphate buffer, and ammonium sulfate solutions as collagen solvents; dialyzing the resulting collagen solutions against solutions of various electrolyte salts; and finally reconstituting the dissolved collagen by either exhaustive dialysis removal of solvent, or shift in pH. The precipitated collagen fibrils showed different solubility characteristics and a large variety of fibrillar forms which seemed to be dependent on previous electrolytic environment.

Dissolving of collagen in the above cases is probably brought about by the breaking of inter-molecular hydrogen bonds because of the presence of the inorganic ions so that large collagen fibres go into solution as collagen molecules. These are relatively stable because the sites of the previous inter-molecular hydrogen bonds are now available to form intra-molecular hydrogen bonds.

In solution there is probably extensive binding of buffer ions to the collagen molecules. The bound ions can block off sites that would normally lead to one type of cross-linking, and in turn create new sites which produce their own characteristic cross-linking. Ion binding also affects the charge pattern of the collagen molecule in solution so as to alter the time-average multipole that arises from fluctuations in charge. These combined effects could account for the variety of fibrillar forms that are reconstituted from the collagen solutions.

The progressive insolubility of collagen as it is repeatedly reconstituted and dissolved may be due to the fact that the bound ions form inter-molecular salt bridges that are very difficult to break. This, plus the fact that dialysis to exhaustion of buffer salts from a collagen solution does not completely erase the effects of previous contact with a given electrolyte, indicate that bound ions tend to form a stable system.

CHROMATOGRAPHIC SEPARATION OF PROTEINS AND PEPTIDES

by
Norbert J. Hipp
Eastern Utilization Research and Development Division

The literature on the chromatography of proteins and peptides is extensive and reviews appear each year in not one but a number of places. After a systematic study (1) of resin-protein properties, a most important stimulus to protein chromatography was provided by Hirs, Stein, Tallan and Moore (2) who showed that the basic enzymatic proteins, ribonuclease and lysozyme, could be successfully prepared and analyzed chromatographically on the carboxylic acid cation exchanger IRC-50. Hirs et al. (2), using elution analysis, the most advantageous form of chromatography, found that ribonuclease, homogeneous by electrophoresis and ultracentrifugation, contained two enzymatically active, chromatographically distinct components. Cole (3) has successfully chromatographed the non-basic protein insulin on IRC-50 using urea to disrupt multiple hydrogen bonding so that simple ion exchange could take place.

Hirs (4) described the use of Dowex 50-2X for the resolution of peptides obtained by enzymatic hydrolysis of oxidized ribonuclease which enabled the authors to establish the sequence of amino acids in ribonuclease. Dowex 50-8X and Dowex 1-10X was used in a study of the phosphopeptides obtained by a partial acid hydrolysis of α-casein (5). Sober and Peterson introduced ion exchange materials prepared from cellulose (6) and demonstrated the fractionation of human serum on DEAE cellulose (7). Remarkable separation of the egg white proteins were obtained on CM cellulose (8) and on DEAE cellulose (9). Groves (10) used DEAE cellulose for the isolation of a red protein from milk. A new material known as Sephadex, a cross-linked dextran, was introduced by Porath and Flodin (11). The degree of cross-linkage of the gel determines the rate at which a given molecular weight material will pass through a column. Low molecular weight material will enter the gel structure and be retarded on passage through the column and will be eluted later in the chromatogram than the high molecular weight material. Qualitative information on the interaction of dextran gels with proteins, peptides, amino acids and some amino acid derivatives was recently reported by Porath (12). The utility of Sephadex is apparent and extensive use of this material may be anticipated.

References

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AMINO ACID ANALYSIS OF MILK PROTEINS

by
W. G. Gordon
Eastern Utilization Research and Development Division

The first complete amino acid analysis of a large protein was that of β-lactoglobulin by Brand et al. (1) in 1945. Many different methods of analysis, including chemical micro methods, specific enzyme procedures and microbiological assays, were needed for this accomplishment. However, only 2.5 grams of protein were used in the analysis. This classical work ushered in a new era in protein chemistry because it demonstrated the usefulness of the newly-introduced microbiological assay procedures for amino acids and also that an amino acid analysis could be completed on relatively small amounts of protein.

Some years later, Gordon et al. (2,3,4), using many of the same techniques employed by Brand, determined the amino acid composition of whole casein and of its principal components, alpha-, beta-, and gamma-caseins. Significant differences in composition, which could be related to differences in physico-chemical properties, were found. In these investigations a single period of hydrolysis, namely, 20 hours at 120°, was used in most of the analyses and the factors of Rees, which had been worked out for mixtures of amino acids, were applied arbitrarily to the results for serine and threonine in order to correct for destruction during acid hydrolysis. The time required for a complete amino acid analysis by these methods could be estimated in man-years.

Our next analysis of a purified milk protein was done on α-lactalbumin (5). The elegant method of chromatography on columns of ion-exchange resin which had been described by Moore and Stein (6) was the method of choice for precise amino acid analysis of the crystalline protein. However, tryptophan and cystine were determined by other methods. Hydrolysates for chromatography were prepared after heating for 20, 70, and 140 hours. It was anticipated that there would be progressive destruction of serine and threonine with increasing time of hydrolysis. In the case of α-lactalbumin such destruction did not occur, but tyrosine values did decrease progressively. At the same time, the longer periods of hydrolysis were required for maximal yields of valine and isoleucine, in agreement with similar findings by other investigators on other proteins, for example, insulin (7). Outstanding features of the amino acid composition of α-lactalbumin were its high content of aspartic acid and tryptophan and low content of arginine, methionine and proline. Only a few hundred milligrams of protein were needed in this work. A single complete analysis could be made on about 10 mg. protein in approximately one week.

More recently, it became desirable to re-investigate the amino acid composition of alpha-casein. Not only was alpha-casein itself further purified, but methods were developed for the sub-fractionation of alpha-casein. We have analyzed purified alpha-casein and its major sub-fraction, alpha$_1$-casein (8), as well as two other sub-fractions, alpha$_2$ - or lambda-casein and alpha$_3$-casein, which may be similar to kappa-casein (9). The improved chromatographic procedure of Moore Spackman and Stein (10) was employed for these analyses. The results, which

have not yet been published, show good agreement between the newer analyses and our previous analyses of alpha-casein. Alpha$_1$-casein is similar in amino acid content to alpha-casein, as might be expected, because it is the major sub-component. Alpha$_3$-casein is significantly different in composition from alpha$_1$-casein while alpha$_2$-casein appears to be intermediate in composition between alpha$_1$- and alpha$_3$-caseins. This chromatographic system for amino acid analysis was found to be excellent, requiring only about 4 mg. of protein and 48 hours for a complete analysis.

At present we are using an automatic recording apparatus for amino acid analysis (11). This employes essentially the same chromatographic techniques as the fraction-collector method (10) but an analysis can be made in only 24 hours. In a study of the amino acid composition of the genetically different β-lactoglobulins A and B, it has been found that the two forms, although very closely related chemically, differ in content of aspartic acid, glycine, alanine and valine (12). The differences are small and might not have been demonstrable were it not for the high reproducibility inherent in the automatic system.

References

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ALLERGENIC PROTEINS OF OILSEEDS

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A brief description of the origin and organization of what is now the Allergens Laboratory of the Eastern Utilization Research and Development Division was given. Some basic principles of allergy and immunology, some definitions and the cutaneous and passive transfer tests used in the work were described.

The isolation and chemical and immunological properties of the principal allergen of cottonseed, CS-1A, were described. This isolation procedure was generalized by its application to other oilseeds and nuts which form a distinct clinical grouping. Almond nuts, Brazil nuts, castor beans, Barcelona and DuChilly filbert nuts, flaxseed, kapok seed and mustard seed yielded allergens chemically similar to CS-1A, but possessing their own specificities. Coconut, pecan nuts, tung nuts and English walnuts yielded no allergen by the CS-1A procedure. Peanuts, soy beans and black walnuts yielded a fraction chemically similar to CS-1A, but immunologically atypical. These allergens were classified as natural proteoses. They have the following properties: soluble in water and basic lead acetate solution, insoluble in 75% ethanol, stable to boiling water, partially dialyzable, composed of amino acids and characterized by realtively high arginine and glutamic acid contents, contain chemically combined polysaccharide, but allergenic and antigenic specificities are due to the protein components; they are immunologically distinct from other allergens and antigens in respective seeds or nuts.

The effects of the proteolytic enzymes, trypsin, chymotrypsin, pepsin and carboxypeptidase on the cottonseed allergen were discussed. Trypsin and chymotrypsin followed by trypsin destroyed allergenic activity of CS-1A. Pepsin and carboxypeptidase partially hydrolized CS-1A and the split products retained allergenic and antigenic activity.

Dialysis and chromatographic fractionation of CS-1A with Amberlite IRC-50 (XE-64) yielded two active fractions which were demonstrably free from each other.

A quantitative, passive transfer method using serum from a cottonseed sensitive person was devised to critically evaluate and compare these separated allergenic fractions. The method determined and compared: (1) the passive transfer inciting capacities, (2) the reagin neutralizing capacities, and (3) whether or not the specificities of two allergenic fractions were the same. The reproducibility of the method was such that twofold differences in quantity of allergen could be determined. Conclusive, confirmatory evidence was obtained with this method showing that CS-1A was a complex mixture of proteins and polysaccharidic proteins, essentially, all possessing the same allergenic specificity. It was shown by this method that 10-11 and 10-12 molar solutions of allergen could be detected in human test subjects.

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